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CTLA-4 and PD-1 Pathways

Similarities, Differences, and Implications of Their Inhibition

Elizabeth I. Buchbinder, MD* and Anupam Desai, MD†

Abstract: The cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed death 1 (PD-1) immune checkpoints are negative regulators of T-cell immune function. Inhibition of these targets, resulting in increased activation of the immune system, has led to new immunotherapies for melanoma, non-small cell lung cancer, and other cancers. Ipilimumab, an inhibitor of CTLA-4, is approved for the treatment of advanced or unresectable melanoma. Nivolumab and pembrolizumab, both PD-1 inhibitors, are approved to treat patients with advanced or metastatic melanoma and patients with metastatic, refractory non-small cell lung cancer. In addition the combination of ipilimumab and nivolumab has been approved in patients with BRAF WT metastatic or unresectable melanoma. The roles of CTLA-4 and PD-1 in inhibiting immune responses, including antitumor responses, are largely distinct. CTLA-4 is thought to regulate T-cell proliferation early in an immune response, primarily in lymph nodes, whereas PD-1 suppresses T cells later in an immune response, primarily in peripheral tissues. The clinical profiles of immuno-oncology agents inhibiting these 2 checkpoints may vary based on their mechanistic differences. This article provides an overview of the CTLA-4 and PD-1 pathways and implications of their inhibition in cancer therapy.

Key Words: cytotoxic T-lymphocyte-associated antigen 4, CTLA-4, programmed death 1, PD-1, immune checkpoint

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A key requirement of the immune system is to distinguish self from nonself. While the concept is simple, the implementation is a complex system that has taken decades to understand. At the center of this process is recognition and

binding of a T-cell receptor (TCR) to an antigen displayed in the major histocompatibility complex (MHC) on the surface of an antigen-presenting cell (APC). Multiple other factors then influence whether this binding results in T-cell activation or anergy.

The life of a T cell begins in the thymus, where immature cells proliferate and create a wide repertoire of TCRs through recombination of the TCR gene segments. A selection process then begins, and T cells with strong reactivity to self-peptides are deleted in the thymus to prevent autoreactivity in a process called central tolerance.¹ T cells with insufficient MHC binding undergo apoptosis, but those that can weakly respond to MHC molecules and self-peptides are not deleted and are released as naive cells to circulate through the blood, spleen, and lymphatic organs. There they are exposed to professional APCs displaying foreign antigens (in the case of infection) or mutated self-proteins (in the case of malignancy). Some TCRs may have specificity that is cross-reactive with self-antigens. To prevent autoimmunity, numerous immune checkpoint pathways regulate activation of T cells at multiple steps during an immune response, a process called peripheral tolerance.^{1,2} Central to this process are the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed death 1 (PD-1) immune checkpoint pathways.³ The CTLA-4 and PD-1 pathways are thought to operate at different stages of an immune response. CTLA-4 is considered the “leader” of the immune checkpoint inhibitors, as it stops potentially autoreactive T cells at the initial stage of naive T-cell activation, typically in lymph nodes.^{2,4} The PD-1 pathway regulates previously activated T cells at the later stages of an immune response, primarily in peripheral tissues.² A core concept in cancer immunotherapy is that tumor cells, which would normally be recognized by T cells, have developed ways to evade the host immune system by taking advantage of peripheral tolerance.^{5,6} Inhibition of the immune checkpoint pathways has led to the approval of several new drugs: ipilimumab (anti-CTLA-4), pembrolizumab (anti-PD-1), and nivolumab (anti-PD-1). There are key similarities and differences in these pathways, with implications for cancer therapy.

CTLA-4 PATHWAY

T-cell activation is a complex process that requires >1 stimulatory signal. TCR binding to MHC provides specificity to T-cell activation, but further costimulatory signals are required. Binding of B7-1 (CD80) or B7-2 (CD86) molecules on the APC with CD28 molecules on the T cell leads to signaling within the T cell. Sufficient levels of CD28:B7-1/2 binding lead to proliferation of T cells, increased T-cell survival, and differentiation through the production of growth cytokines such as interleukin-2 (IL-2), increased energy metabolism, and upregulation of cell survival genes.

CTLA-4 is a CD28 homolog with much higher binding affinity for B7^{7,8}; however, unlike CD28, binding of CTLA-4 to

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B7 does not produce a stimulatory signal. As such, this competitive binding can prevent the costimulatory signal normally provided by CD28:B7 binding^{7,9,10} (Fig. 1). The relative amount of CD28:B7 binding versus CTLA-4:B7 binding determines whether a T cell will undergo activation or anergy.⁴ Furthermore, some evidence suggests that CTLA-4 binding to B7 may actually produce inhibitory signals that counteract the stimulatory signals from CD28:B7 and TCR:MHC binding.^{11,12} Proposed mechanisms for such inhibitory signals include direct inhibition at the TCR immune synapse, inhibition of CD28 or its signaling pathway, or increased mobility of T cells leading to decreased ability to interact with APCs.^{9,12,13}

CTLA-4 itself is subject to regulation, particularly by localization within the cell. In resting naive T cells CTLA-4 is located primarily in the intracellular compartment.¹⁴ Stimulatory signals resulting from both TCR and CD28:B7 binding induce upregulation of CTLA-4 on the cell surface by exocytosis of CTLA-4-containing vesicles.¹⁴ This process operates in a graded feedback loop whereby stronger TCR signaling elicits more CTLA-4 translocation to the cell surface. In case of a net negative signal through CTLA-4:B7 binding, full activation of T cells is prevented by inhibition of IL-2 production and cell cycle progression.¹⁵

CTLA-4 is also involved in other aspects of immune control. Regulatory T cells (Tregs) control functions of the effector T cells, and thus are key players in maintaining peripheral tolerance.^{16,17} Unlike effector T cells, Tregs constitutively express CTLA-4, and this is thought to be important

for their suppressive functions.¹⁷ In animal models, genetic CTLA-4 deficiency in Tregs impaired their suppressive functions.^{17,18} One mechanism whereby Tregs are thought to control effector T cells is downregulation of B7 ligands on APCs, leading to reduced CD28 costimulation (Fig. 2).^{18,19}

PD-1 PATHWAY

PD-1 is a member of the B7/CD28 family of costimulatory receptors. It regulates T-cell activation through binding to its ligands, programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2).²⁰ Similar to CTLA-4 signaling, PD-1 binding inhibits T-cell proliferation, and interferon- γ (IFN- γ), tumor necrosis factor- α , and IL-2 production, and reduces T-cell survival²⁰ (Fig. 3). If a T cell experiences coincident TCR and PD-1 binding, PD-1-generated signals prevent phosphorylation of key TCR signaling intermediates, which terminates early TCR signaling and reduces activation of T cells.^{10,21} PD-1 expression is a hallmark of “exhausted” T cells that have experienced high levels of stimulation or reduced CD4⁺ T-cell help.²² This state of exhaustion, which occurs during chronic infections and cancer, is characterized by T-cell dysfunction, resulting in suboptimal control of infections and tumors.

Both CTLA-4 and PD-1 binding have similar negative effects on T-cell activity; however, the timing of downregulation, the responsible signaling mechanisms, and the anatomic locations of immune inhibition by these 2 immune checkpoints differ. Unlike CTLA-4, which is confined to

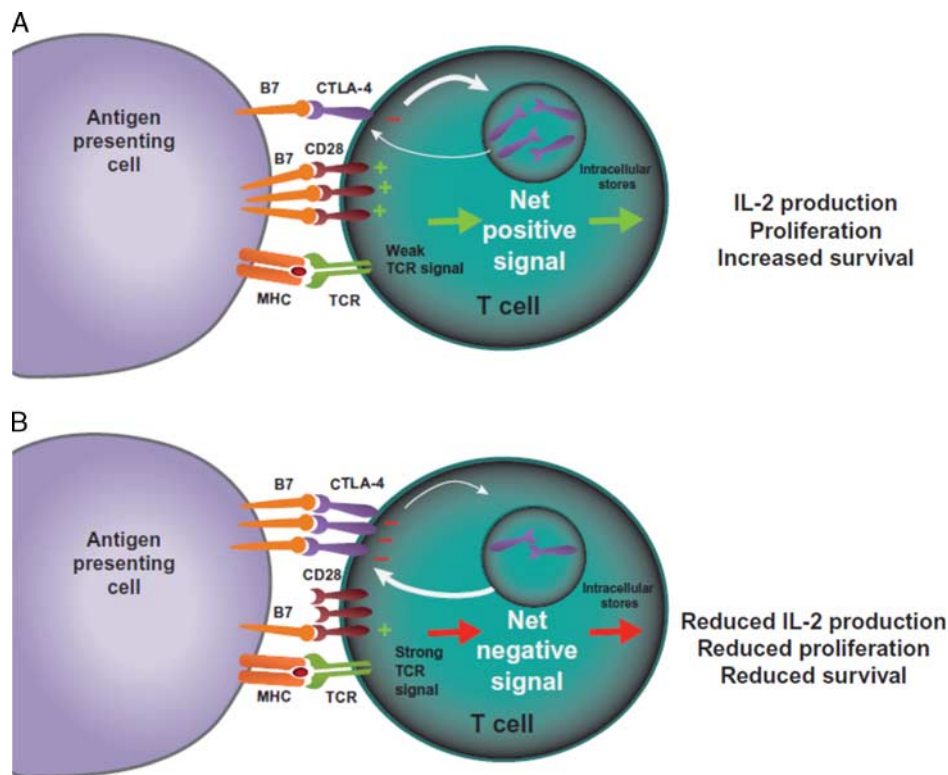


FIGURE 1. CTLA-4-mediated inhibition of T cells. T cells are activated when TCRs bind antigen displayed in the MHC on antigen-presenting cells in concert with CD28:B7-mediated costimulation. A, In the case of a weak TCR stimulus, CD28:B7 binding predominates, resulting in a net positive activating signal and IL-2 production, proliferation, and increased survival. B, In the case of a strong TCR stimulus, CTLA-4 expression is upregulated by increased transport to the cell surface from intracellular stores and decreased internalization. CTLA-4 competes with CD28 for binding of B7 molecules. Increased CTLA-4:B7 binding can result in a net negative signal, which limits IL-2 production and proliferation, and limits survival of the T cell. CTLA-4 indicates cytotoxic T-lymphocyte-associated antigen 4; IL-2, interleukin-2; MHC, major histocompatibility complex; TCR, T-cell receptor.

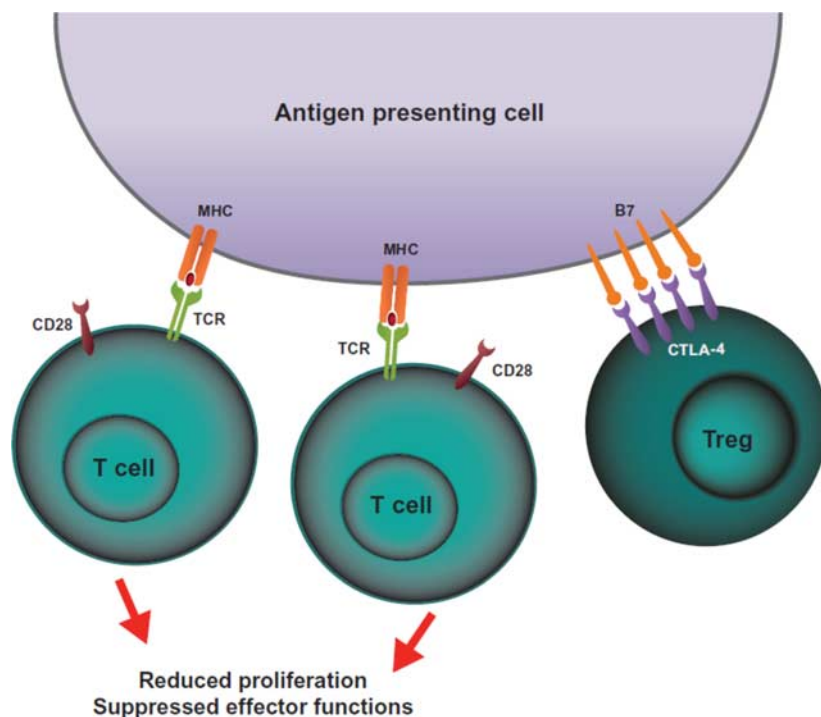


FIGURE 2. CTLA-4-mediated inhibition of Tregs. One hypothesis of how CTLA-4 expression on Tregs can inhibit T-cell activation is depicted. Constitutive expression of CTLA-4 on Tregs can sequester or cause internalization (not depicted) of B7 molecules on antigen-presenting cells. The lack of CD28:B7-mediated costimulation leads to reduced T-cell proliferation and reduced effector functions. CTLA-4 indicates cytotoxic T-lymphocyte-associated antigen 4; MHC, major histocompatibility complex; TCR, T-cell receptor; Tregs, regulatory T cells.

T cells, PD-1 is more broadly expressed on activated T cells, B cells, and myeloid cells.^{2,20} While CTLA-4 functions during the priming phase of T-cell activation, PD-1 functions during the effector phase, predominantly within peripheral tissues.²⁰

The distribution of PD-1 ligands also differs from those for CTLA-4. The B7 ligands for CTLA-4 are expressed by professional APCs, which typically reside in lymph nodes or spleen²; however, PD-L1 and PD-L2 are more widely expressed.^{2,10,23,24} PD-L1 is expressed on leukocytes, on non-hematopoietic cells, and in nonlymphoid tissues, and can be induced on parenchymal cells by inflammatory cytokines (IFN- γ) or tumorigenic signaling pathways.²⁵ PD-L1 expression is also found on many different tumor types, and is associated with an increased amount of tumor-infiltrating lymphocytes (TILs) and poorer prognosis.^{26–28} PD-L2 is primarily expressed on dendritic cells and monocytes, but can be induced on a wide variety of other immune cells and nonimmune cells, depending on the local microenvironment.²⁹ PD-1 has a higher binding affinity for PD-L2 than for PD-L1, and this difference may be responsible for differential contributions of these ligands to immune responses.³⁰ Because PD-1 ligands are expressed in peripheral tissues, PD-1–PD-L1/PD-L2 interactions are thought to maintain tolerance within locally infiltrated tissues.²

As might be expected, the plurality of ligands for PD-1 leads to variation in biological effects, depending upon which ligand is bound. One model showed opposing roles of PD-L1 and PD-L2 signaling in activation of natural killer T cells.³¹ Inhibition of PD-L2 binding leads to enhanced T_H2 activity,³² whereas PD-L1 binding to CD80 has been shown to inhibit T-cell responses.³³ These different biological effects are likely to contribute to differences in activity and toxicity between antibodies directed at PD-1 (preventing binding to both

ligands) as opposed to those directed at PD-L1, and therefore have potential therapeutic implications.

Although Tregs express PD-1 as well as CTLA-4, the function of PD-1 expression on these cells remains unclear. PD-L1 has been shown to contribute to the conversion of naive CD4⁺ T cells to Treg cells³⁴ and to inhibit T-cell responses by promoting the induction and maintenance of Tregs.³⁵ Consistent with these findings, PD-1 blockade can reverse Treg-mediated suppression of effector T cells *in vitro*.³⁶

PD-1 binding with its ligands decreases the magnitude of the immune response in T cells that are already engaged in an effector T-cell response.²² This results in a more restricted spectrum of T-cell activation compared with CTLA-4 blockade, which may explain the apparently lower incidence of immune-mediated adverse events (AEs) associated with PD-1 compared with a CTLA-4 blockade (see below).³⁷ Similarities and differences between the CTLA-4 and PD-1 receptors, and the consequences of their engagement, are detailed in Box 1.

IMPLICATIONS OF CTLA-4 AND PD-1 PATHWAY BLOCKADE IN CANCER

Preclinical studies showing decreased tumor growth and improved survival with CTLA-4 or PD-1 pathway blockade provide the rationale for immune checkpoint inhibition for cancer treatment.^{39,40} Monoclonal antibodies that block CTLA-4 or PD-1 are now approved for melanoma and lung cancer, and are in development for other tumor types, including kidney cancer, prostate cancer, and head and neck cancer (Table 1).^{41–44} Other agents targeting PD-L1 specifically are also in development (Table 1).^{41–44}

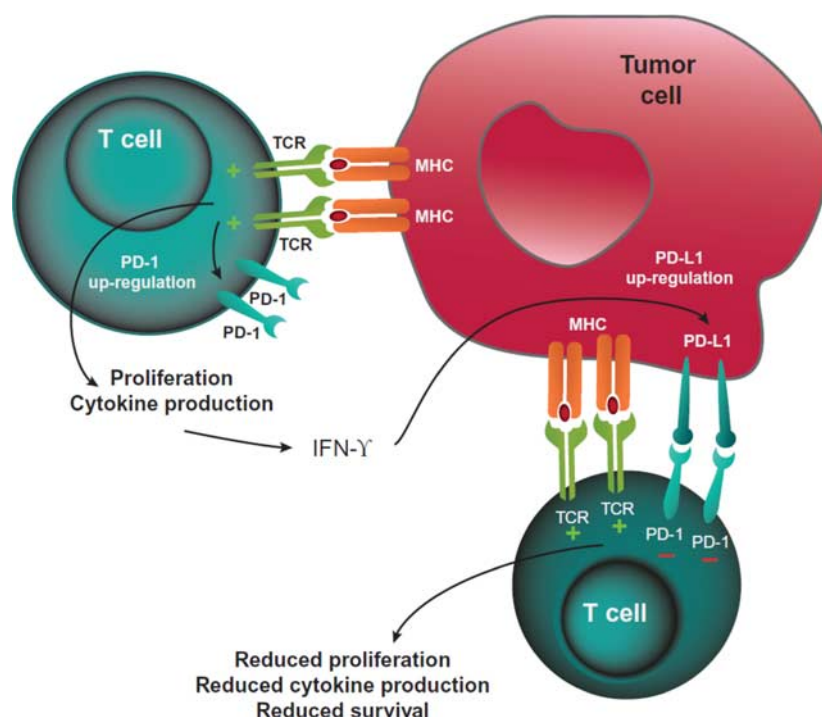


FIGURE 3. PD-1-mediated inhibition of T cells. T cells recognizing tumor antigens can be activated to proliferate, secrete inflammatory cytokines, and resist cell death. Prolonged TCR stimulation during an ongoing immune response can cause upregulated PD-1 expression. Tumor cells can express PD-L1 (and PD-L2, not shown) as a consequence of inflammatory cytokines and/or oncogenic signaling pathways. PD-1:PD-L1 binding inhibits TCR-mediated positive signaling, leading to reduced proliferation, reduced cytokine secretion, and reduced survival. IFN- γ indicates interferon- γ ; MHC, major histocompatibility complex; PD-1, programmed death protein 1; PD-L1, programmed death ligand 1; PD-L2, programmed death ligand 2; TCR, T-cell receptor.

The exact mechanism by which anti-CTLA-4 antibodies induce an antitumor response is unclear, although research to date suggests that CTLA-4 blockade affects the immune priming phase by supporting the activation and proliferation of a higher number of effector T cells, regardless of TCR specificity, and by reducing Treg-mediated suppression of T-cell responses (Fig. 4).² An increase in the diversity of the peripheral T-cell pool following CTLA-4 blockade in patients with melanoma has recently been reported.⁴⁵ An ipilimumab study in patients with melanoma or prostate cancer provided

evidence that baseline T-cell profile may also be important. An immediate turnover of the T-cell repertoire on initial treatment was shown, and it continued to evolve with further treatment; both expansion and loss of individual T-cell clonotypes were identified, but there was a net increase in TCR diversity.⁴⁶ Overall survival, however, was associated with the maintenance of clones present in high frequency at baseline. In patients with shorter overall survival, numbers of these highest frequency clones decreased with treatment. These findings suggest that effective CTLA-4 blockade may depend on the

Box 1. A Comparison of CTLA-4 and PD-1

Similarities

- B7 receptor family members³
- Expressed by activated T cells^{14,20}
- Level of expression affected by the strength and/or duration of TCR signaling^{20,22,38}
- Regulate an overlapping set of intracellular T-cell signaling proteins¹⁰
- Reduce T-cell proliferation, glucose metabolism, cytokine production, and survival^{2,10,20}

Differences

- CTLA-4 limits T-cell responses early in an immune response, primarily in lymphoid tissues; PD-1 limits T-cell responses later in an immune response, primarily in peripheral tissues²
- CTLA-4 expressed by T cells; PD-1 expressed by T cells and other immune cells^{2,20}
- CTLA-4 ligands expressed by professional antigen-presenting cells; PD-1 ligands expressed by antigen-presenting cells and other immune cells, and can be inducibly expressed on nonimmune cells, including tumor cells^{2,20}
- PD-1 engagement interferes with more T-cell signaling pathways than does CTLA-4 engagement¹⁰
- CTLA-4 affects Treg functioning; the role of PD-1 on Tregs is unclear^{2,17,18,20}

CTLA-4 indicates cytotoxic T-lymphocyte-associated antigen 4; PD-1, programmed death protein 1; TCR, T-cell receptor; Treg, regulatory T cell.

TABLE 1. CTLA-4 and PD-1 Pathway Inhibitors Approved or in Phase II and/or III Clinical Trial Stage of Development^{41–44}

Target	Name	Status*	Company
CTLA-4	Ipilimumab	Approved for the treatment of unresectable or metastatic melanoma Phase III: lung cancer, kidney cancer, and prostate cancer Phase II: cervical cancer, colorectal cancer, gastric cancer, pancreatic cancer, ovarian cancer, and urothelial cancer	Bristol-Myers Squibb
CTLA-4	Tremelimumab	Phase II studies in lung cancer	MedImmune/ AstraZeneca
PD-1	Pembrolizumab	Approved in the United States for treatment of unresectable or metastatic melanoma [†] Phase III: gastric/GEJ cancer, lung cancer, head and neck cancer, and urothelial cancer Phase II: colorectal cancer, glioblastoma, Merkel cell cancer, pancreatic cancer, and hematologic malignancies	Merck
PD-1	Nivolumab	Approved in the United States for second-line/third-line treatment of unresectable or metastatic melanoma [†] and for the treatment of metastatic non–small cell lung cancer [‡] Phase III: gastric cancer, glioblastoma, head and neck cancer, kidney cancer, and lung cancer (nonsquamous) Phase II: cervical cancer, colorectal cancer, pancreatic cancer, and hematologic malignancies	Bristol-Myers Squibb
PD-1	Pidilizumab	Phase II: kidney cancer and hematologic malignancies	CureTech/ Medivation
PD-L1	Durvalumab	Phase III: head and neck cancer and lung cancer Phase II: colorectal cancer and glioblastoma	MedImmune/ AstraZeneca
PD-L1	Atezolizumab	Phase III: bladder cancer and lung cancer Phase II: kidney cancer	Roche

*Only most advanced phase of development for any tumor type is listed; phase I or phase I/II indications are not listed. Includes both monotherapy and combination trials. Information from clinicaltrials.gov.

[†]With disease progression following ipilimumab and, if *BRAF* V600 mutation positive, a *BRAF* inhibitor. Or in combination with ipilimumab in *BRAF* WT patients.

[‡]With disease progression on or after platinum-based chemotherapy. Patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving pembrolizumab or nivolumab.

CTLA-4 indicates cytotoxic T-lymphocyte–associated antigen 4; GEJ, gastroesophageal junction; PD-1, programmed death 1; PD-L1, programmed death ligand 1.

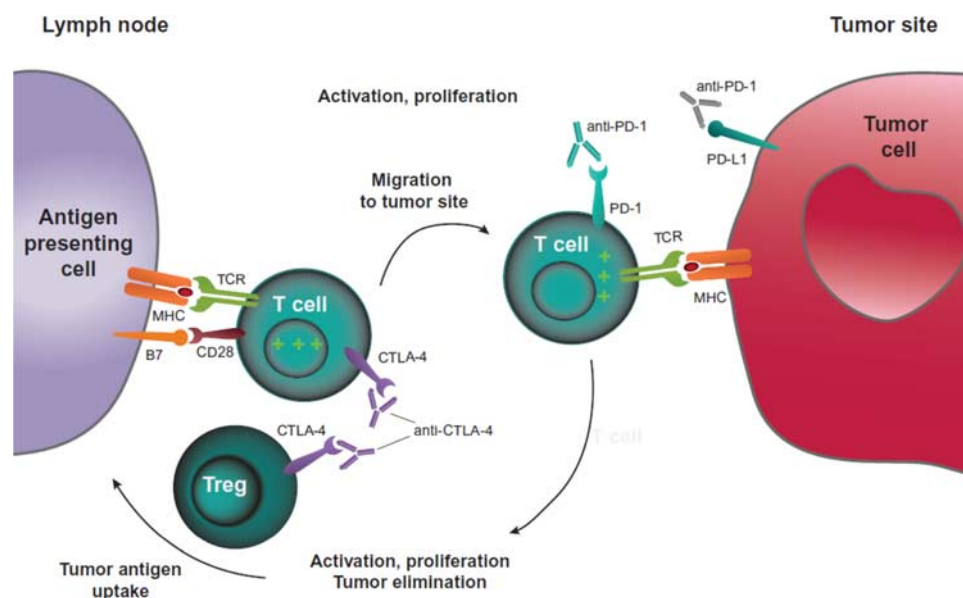


FIGURE 4. CTLA-4 and PD-1 pathway blockade. CTLA-4 blockade allows for activation and proliferation of more T-cell clones, and reduces Treg-mediated immunosuppression. PD-1 pathway blockade restores the activity of antitumor T cells that have become quiescent. A dual pathway blockade could have a synergistic effect, resulting in a larger and longer lasting antitumor immune response. CTLA-4 indicates cytotoxic T-lymphocyte–associated antigen 4; MHC, major histocompatibility complex; PD-1, programmed death 1; PD-L1, programmed death ligand 1; TCR, T-cell receptor; Treg, regulatory T cell.

ability to retain preexisting high-avidity T cells with relevance to the antitumor response.

PD-1 blockade works during the effector phase to restore the immune function of T cells in the periphery that have been turned off following extended or high levels of antigen exposure, as in advanced cancer.^{22,23} As mentioned above, the ligands for PD-1 can be expressed by tumor cells as well as tumor-infiltrating immune cells. PD-L1 expression on tumor cells varies by tumor type and also within a given tumor type, but appears to be particularly abundant in melanoma, non-small cell lung cancer (NSCLC), and ovarian cancer.^{27,28,47} In a recent study, PD-L1 expression on tumor cells was shown to be significantly associated with PD-1 expression on TILs, and was locally associated with PD-L2 expression when this ligand was also expressed.²⁷ In the same study, tumor PD-L1 expression was the single factor most closely correlated with response to anti-PD-1 blockade, whereas PD-L1 expression on TILs was not associated with response.²⁷ Another study, however, found that patient response to anti-PD-L1 blockade was strongest when PD-L1 was expressed by tumor-infiltrating immune cells.⁴⁸

Inhibiting PD-L1 specifically, as opposed to PD-1 inhibition, will block PD-1:PD-L1 interactions while preserving PD-1:PD-L2 interactions. This has the potential to provide a more targeted signal with less unwanted toxicity, as self-tolerance mediated through PD-1:PD-L2 interactions should be preserved.^{37,49} Furthermore, as PD-L1 is known to bind CD80 as well as PD-1 to deliver inhibitory signals to T cells,³³ PD-L1 inhibition with an appropriate antibody could in theory also prevent PD-L1 reverse signaling and its resulting T-cell downregulation through CD80; a PD-L1-directed antibody could also interrupt the PD-L1:CD80 axis on other cells where they are coexpressed, such as dendritic cells.^{20,23}

The differences in timing, location, and nonredundant effects of their actions suggest that anti-CTLA-4–targeted therapies and anti-PD-1 therapies have the potential for additive or possibly synergistic effects in the treatment of advanced malignancy. Further evidence that supports this theory and highlights the different role of each immune checkpoint comes from a study that investigated the biological effect of CTLA-4 and PD-1 blockade in patients undergoing single-agent or combination treatment.⁵⁰ While CTLA-4 inhibition induced a proliferative signal found predominantly in a subset of transitional memory T cells, PD-1 inhibition was associated with changes in genes thought to be involved in cytotoxicity and natural killer cell function; dual blockade led to nonoverlapping changes in gene expression. The 2 treatment types also produced different effects on levels of circulating cytokines. This study confirms that CTLA-4 and PD-1 blockade lead to distinct patterns of immune activation, supporting the rationale for the investigation of immune checkpoint combinations in the clinic.

CLINICAL EFFICACY AND CHARACTERISTICS OF RESPONSES WITH IMMUNE CHECKPOINT INHIBITORS

Anti-CTLA-4 blockade with ipilimumab was the first treatment to prolong overall survival in patients with advanced melanoma in a randomized setting.^{51,52} Analysis of long-term survival data pooled across several phase II and phase III trials showed that the survival curve begins to plateau at about 3 years, with 3-year survival rates of 22%, 26%, and 20% in all patients with sufficient follow-up, in treatment-naïve patients, and in previously treated patients, respectively.⁵³ Consistent with its survival benefit, CTLA-4 blockade is associated with

lasting responses in a proportion of patients treated, with some responses reported to last >3 years.^{51,54}

More recently, PD-1 blockade has been shown to improve survival and progression-free survival in patients with metastatic melanoma and in patients with previously treated metastatic squamous and nonsquamous NSCLC.^{55–62} The longest follow-up data available indicate that highly durable responses can also occur with PD-1 blockade in patients with melanoma, NSCLC, or renal cell carcinoma (RCC).^{48,63–66} The response rates with PD-1 pathway blockade were higher than with CTLA-4 blockade in advanced melanoma: 33% to 34% versus 12% of patients in a phase III head-to-head trial of pembrolizumab versus ipilimumab. This trial also reported higher 1-year survival rates with pembrolizumab versus ipilimumab: 68% to 74% versus 58%.⁵⁴

Because immune checkpoint inhibitors work by restarting an effective antitumor immune response, response patterns can differ from those seen with chemotherapy or targeted agents. Delayed or unconventional responses may be related to variations in the kinetics and efficacy of each patient's individual immune system, as well as its interplay with tumors and metastases. An initial increase in target lesion tumor volume could be because of true tumor growth before the generation of effective antitumor response. Conversely, faster activation of an antitumor immune response could lead to inflammation and an influx of immune cells into the tumor site, which could masquerade as tumor progression. In clinical trials of ipilimumab, approximately 10% of patients were initially characterized as having progressive disease by World Health Organization criteria, but subsequently had favorable survival.⁶⁷ Approximately 4% to 8% of patients with advanced melanoma receiving nivolumab or pembrolizumab in clinical trials had unconventional responses that did not meet Response Evaluation Criteria in Solid Tumors (RECIST) criteria, but were nevertheless associated with patient benefit.^{55,66,68,69} Unconventional response patterns have also been observed in patients with lung cancer or RCC receiving PD-1 pathway inhibitors.^{56,64,65,70} These atypical responses have led to the development of modified response criteria called immune-related response criteria (Supplemental Table 1, Supplemental Digital Content 1, <http://links.lww.com/AJCO/A110>^{67,68,71}).

BIOMARKERS

A frustration with ipilimumab has been the inability to predict prospectively which patients are most likely to benefit from treatment. The low level of inducible CTLA-4 expression and the widespread expression of its B7 ligands are not useful as predictive biomarkers. Retrospective studies have identified several markers associated with response, including absolute lymphocyte count, upregulation of the T-cell activation marker-inducible costimulator (ICOS), and the development of a polyfunctional T-cell response to the tumor antigen NY-ESO-1.⁷² To date, none of these potential markers have been validated prospectively. An association between melanoma mutational load and clinical benefit with CTLA-4 blockade has been shown, but was insufficient alone to predict patients who are likely to respond to treatment⁷³; however, work examining tumor neoantigens has shown promise, with the identification of a neoantigen signature present in tumors that correlated with overall survival of individuals treated with CTLA-4 blockade.⁷³

In contrast, the upregulation of PD-1 on exhausted cells and of PD-L1 ligands on tumor cells or tumor-infiltrating immune cells may offer the potential for identifying patients

responsive to PD-1 or PD-L1 blockade.^{27,48} Preliminary data across tumor types suggest that patients with PD-L1-expressing tumors or infiltrating immune cells typically have a higher response rate to anti-PD-1 or anti-PD-L1 therapy and may also have improved survival outcomes compared with patients with low or negative PD-L1 expression.^{27,48,55,63,64,70,74,75} However, in most studies, responses have also been seen in patients with PD-L1-low or PD-L1-negative tumors, and thus these patients should not be excluded from treatment. In a trial comparing the combination of ipilimumab and nivolumab against each agent alone, responses in PD-L1-positive patients were similar with the combination versus nivolumab alone, whereas PD-L1-negative patients did better receiving the combination.⁵⁸ While all of these results are provocative, more research is needed to establish the validity and utility of PD-L1 expression as a predictive biomarker.

Other markers of response to anti-PD-1 or PD-L1 therapy have also been explored and include features associated with PD-L1-mediated suppression of preexisting immunity.^{48,76} As with CTLA-4, mutational burden and higher neoantigen burden have recently been shown to be associated with efficacy in patients with NSCLC treated with PD-1 blockade.⁷⁷

IMMUNOLOGIC TOXICITIES

Immune checkpoint blockade is associated with AEs with potential immunologic etiologies, so-called immune-mediated AEs. Commonly reported immune-mediated AEs include rash or pruritus, gastrointestinal disorders, and endocrinopathies.^{54,55,57,66,69,78,79}

The overall rate of grade ≥ 3 AEs was higher with ipilimumab (20%) compared with pembrolizumab (10% to 13%) in a phase III trial.⁵⁴ Theoretically, this could be a consequence of a greater magnitude of T-cell proliferation or reduced Treg-mediated immunosuppression with CTLA-4 blockade, or activation of a smaller number of T-cell clones with PD-1 blockade.

Hypophysitis is reported in about 2% to 4% of patients receiving ipilimumab but in $<1\%$ of patients receiving PD-1 inhibitors^{51,54,55,69,80}; however, this variation in incidence may not be related to differences in immune mechanism of action, but may be explained by ectopic expression of CTLA-4 in the pituitary gland, leading to ipilimumab binding to endocrine cells, followed by complement fixation and inflammation.⁸⁰

Inhibiting PD-L1 rather than PD-1 may result in a slightly different toxicity profile, although clinical data are currently limited. Treatment-related grade 3-4 AEs were reported in 4% to 13% of patients receiving PD-L1 inhibitors in phase I/II trials across 2 different agents and multiple tumor types.^{48,74,75,81} While data from comparative trials are not yet available, the incidence of grade 3-4 treatment-related AEs may trend lower with PD-L1 inhibitors than with PD-1 inhibitors; however, the immune-mediated AEs reported to date have been similar between the 2 types of agents.

BLOCKADE OF BOTH CTLA-4 AND PD-1/PD-L1

Blockade of both CTLA-4 and PD-1 or PD-L1 could, in theory, induce proliferation of a higher number of T cells early in an immune response, restore immune responses of previously activated T cells that have become exhausted, and reduce Treg-mediated immunosuppression (Fig. 4). Preclinical studies showed enhanced antitumor responses using dual blockade compared with single-agent blockade, which was also observed in initial clinical trials.⁸²⁻⁸⁵ This synergistic effect validates the different roles these agents play in immune regulation.

An increased response rate and improved progression-free survival were reported with the ipilimumab-nivolumab combination when compared with ipilimumab alone in a randomized phase III trial in treatment-naïve patients with metastatic melanoma.⁵⁸ The objective response rate was 58% versus 19%, and the median progression-free survival was 11.5 versus 2.9 months for the combination and monotherapy, respectively.⁵⁸ Combinations of CTLA-4 and PD-1 inhibitors are also being investigated in patients with several other tumor types, including advanced NSCLC and RCC. In metastatic RCC, preliminary data suggest that the objective response rate is higher with a combination blockade (38% to 43%) than was seen with PD-1 inhibition alone in a different trial (20% to 22%).^{70,86} Early data from lung cancer trials do not suggest increased antitumor activity with a combination blockade in NSCLC^{87,88}; however, increased antitumor activity was seen with a combination blockade in small cell lung cancer (SCLC) versus nivolumab.⁸⁹

Combining CTLA-4 and PD-1 blockade with the aim of increasing efficacy is highly desirable, but combination treatment could prove more toxic. In patients with previously untreated melanoma or recurrent SCLC, the incidence of drug-related grade 3-4 AEs was 54% to 55% with concurrent blockade compared with 24% to 27% with ipilimumab alone and 15% to 16% with nivolumab alone.^{58,83,89} Prior CTLA-4 inhibition does not appear to predispose patients to development of immune-mediated AEs with PD-1 inhibition,^{57,90,91} which may therefore support sequential rather than combination treatment.

CONCLUSIONS

The CTLA-4 and PD-1 immune checkpoint pathways downregulate T-cell activation to maintain peripheral tolerance, and can be exploited by tumors to induce an immunosuppressive state that allows the tumors to grow and develop instead of being eliminated by the immune system. The differential patterns of the CTLA-4 and PD-1 ligand expression—found primarily in lymphoid tissue and in peripheral tissues, respectively—are central to the hypothesis that CTLA-4 acts early in tolerance induction and PD-1 acts late to maintain long-term tolerance. Inhibitors of CTLA-4 and PD-1 or its ligand, PD-L1, can restore antitumor immune responses, leading to long-term benefit in a substantial proportion of treated patients. As a likely result of their mechanism of action, immune checkpoint inhibitors are associated with immune-mediated toxicities, most of which can be managed successfully with corticosteroids. Preliminary data suggest that simultaneous blockade of both CTLA-4 and PD-1 pathways leads to increased efficacy over CTLA-4 or PD-1 inhibition alone or in sequence, providing additional evidence of the separate roles of these checkpoints in regulating antitumor immune responses. Further trials are needed to confirm these data and validate a combination strategy.

To date, 3 immune checkpoint inhibitors have been approved for use in melanoma; 2 of the 3 are also approved for lung cancer. These and other investigational CTLA-4, PD-1, and PD-L1 inhibitors are in active clinical development for multiple indications and have the potential to revolutionize future treatment options for many patients with advanced cancer.

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